IMMUNOHISTOCHEMICAL DETECTION OF H.PYLORI AND ITS ASSOCIATION WITH SUBTYPES OF GASTRIC CARCINOMAS

this dissertation is submitted to the
Tamil Nadu Dr.M.G.R. Medical University, Chennai
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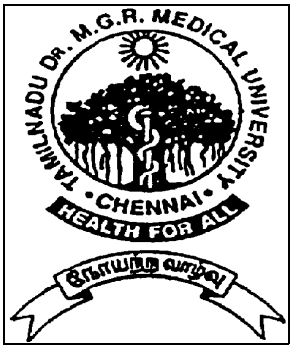

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DECLARATION

I, **Dr.V.JAYANTHI,** solemnly declare that the dissertation titled **"IMMUNOHISTOCHEMICAL DETECTION OF H.PYLORI AND ITS ASSOCIATION WITH SUBTYPES OF GASTRIC CARCINOMAS"** was done by me at Government Stanley Medical College, Chennai – 1, during the academic year 2005 – 2008 under the guidance and supervision of **Prof.A.SUNDARAM. M.D.,** Professor & Head Of the Department, Department of Pathology.

This dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai towards the partial fulfillment of requirement for the award of M.D. Pathology degree.

Place : Chennai **Dr. V. JAYANTHI**

Date : 19.11.2007

CERTIFICATE

This is to certify that this dissertation on **"IMMUNOHISTOCHEMICAL DETECTION OF H.PYLORI AND ITS ASSOCIATION WITH SUBTYPES OF GASTRIC CARCINOMAS"** is a bonafide work done by **Dr. V.JAYANTHI** under my guidance and supervision, appearing for **M.D. Pathology** degree examination in March 2008.

Dr. MYTHILI BHASKARAN M.D.
Dean,
Govt. Stanley Medical College
& Hospital,
Chennai – 600 001.

Prof. A.SUNDARAM, M.D.
Prof. & Head of the Department,
Department of Pathology,
Govt. Stanley Medical College,
Chennai – 600 001.

Place : Chennai

Date : 19 -11-2007

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INTRODUCTION

Gastric carcinoma is estimated to be the second most common cancer in the world.[1]. The incidence of gastric carcinoma shows marked variations from place to place.

INDIA	-	8.9/100000
USA	-	10/100000
JAPAN	-	79.9/100000
ENGLAND	-	18.5/100000

These epidemiological data make it clear that gastric carcinoma incidence is determined by the environmental factors.[1]

Incidence of gastric carcinoma stomach in Government Stanley Medical College Hospital, Chennai is the following

Year	**Total number of malignancies**	**Ca Stomach**
2005	1117	217
2006	1110	210

Stomach is the commonest site for cancer in our hospital. Till recently research on the environmental causes of gastric carcinoma focused primarily on diet. The identification of Helicobactor pylori in chronic inflammatory conditions of the stomach has stimulated interest in its potential role in carcinogenesis. Infection with H.pylori is very common througout the world, occurring in 40-50% of the population in developed countries and 80-90% of the population in developing countries.[2]

High rates of H.pylori infection have also been found in patients with cancer or precancerous conditions (especially in chronic atrophic gastritis).

There are many cross sectional and retrospective studies in many parts of the world which give the causal association between H.pylori infection and gastric cancer. But in Tamilnadu, where gastric carcinoma is prevalent, there are no such studies and this study is intended to know the association between H.pylori and subtypes of gastric adenocarcinomas based on their degree of differentiation (grading) and use of Immonohistochemistry in identifying the organism, especially the coccoid forms along with Giemsa staining for H.pylori and to compare the efficacy of Immonohistochemistry and Giemsa stain.

AIMS AND OBJECTIVES

1. To find out the incidence of H.Pylori in gastrectomy specimens using Giemsa stain and Immunohistochemistry (IHC) using (polyclonal antibody to H.Pylori antigen) in patients who were operated for growth stomach.

2. To compare the efficacy of IHC over Giemsa stain in diagnosing H.Pylori in paraffin embedded tissue sections.

3. To analyse H.Pylori's association with respect to subtypes based on degree of differentiation of gastric adenocarcinomas.

4. To analyse H.Pylori's association with respect to age and sex of patients and site of growth within stomach.

REVIEW OF LITERATURE

Gastric carcinoma is the second most common tumour in the world.Over 99% of gastric cancers are adenocarcinomas[3]. Adenocarcinoma of stomach is primarily a disease of older individuals and it is rare under the age of 40 years.

HISTORICAL REVIEW OF HELICOBACTER PYLORI:

The presence of spiral shaped microorganism was reported in 1893, by Bizzozero [4], an Italian pathologist in the canine stomach. Spiral bacteria [5] were demonstrated for the first time in human stomach in 1906. This initial report concerned patients with gastric carcinoma.

Their presence was not really taken up seriously until late 1970's when Barry Marshell, a second year medical internist along with Robin Warren a histopathologist in Royal Perm hospital, Western Australia noted the appearance of spiral bacteria overlying the gastric mucosa and chiefly over the inflammed tissue.

Warren and Barry cultured these organisms in 1982 from 11 patients with gastritis.[6]

Originally these Camphylobacter like organisms were called Campylobacter pyloridis since they resembled other Campylobacters, both morphologically and in guanine-cytosine DNA content. For grammatical reasons, the name was changed to Campylobacter pylori[7] in 1987.

Subsequently it was shown that Campylobacter pylori did not belong to the genus Campylobacter and it differs in 16 Sr RNA sequences and fatty acid content and possession of multiple polar (upto 7) flagella and a new genus name was suggested in 1989 by Goodwin et al.[8] Helicobacter reflects the two morphological appearances of the organisms, helical in vivo and rod like in vitro.

H.Pylori was the first member of new genus and other Helicobacter including H. mustelae (ferrets), H.muridarum (rats and mice), H.felin (cats and dogs) etc have subsequently been described.

Marshall elegantly fulfilled KOCH's postulates [9] for the role of H.Pylori in antral gastritis with self administration of H.Pylori and showed that it could be cured by use of antibiotics and bismuth salts. The association between H.Pylori and peptic ulcers and possibly carcinoma of stomach was initially suggested by Marshall et al.

Key dates in the history of H.Pylori:

1893 – Gastric spiral bacteria reported for the first time in the stomach of dogs. [4]

1906 – Spirochaetes are demonstrated in the human stomach. [5]

1924 – Urease activity in the stomach is reported.[10]

1950 – Urease in patients with gastric ulceration neutralizes gastric acid via the production of ammonia. [11]

1975 – Gastric spirochaetes and gastritis present in 80% of gastric ulcer.[12]

1983 – Campylobacter – like organisms associated with gastritis and possibly peptic ulceration – beginning of modern era. [6]

1985 – Temporal relationship between acquisition of H.Pylori infection and the development of gastritis.[9]

1989 – The genus "Helicobacter"is suggested.[8]

1994 – H.Pylori classified as a Grade I (definite) carcinogen.[13]

1994 – The infection should be eradicated in patients with peptic ulcer.[14]

Many classifications of gastric carcinoma based on tumour location, invasiveness, histological features, and growth patterns exist.

Classifications of gastric carcinoma.[3]

Based on invasiveness gastric carcinoma is classified into two types as early and late.

In early type, invasion is restricted to submucosa, which is further divided into intramucosal and submucosal types. In the late type, invasion of muscularis externa is present.

Based on the degree of differentiation, gastric adenocarcinoma is divided into three types as Well differentiated, Moderately differentiated and Poorly differentiated adenocarcinoma.

In well differentiated adenocarcinoma, well developed tubular glands, with uniform cytological features are present (greater than 95% of tumour composed of glands). In moderately differentiated adenocarcinoma, complex glands with cribriform change are seen (50-95% composed of gland). In poorly differentiated adenocarcinoma, solid nests of cells, single cells, and signet ring cells with variable cytological abnormalities are seen. (49% or less composed of glands).[15]

This system is simple and reproducible, but may be difficult to apply because of morphologic heterogeneity present in many neoplasms.

Based on the histologic features gastric carcinoma is classified by WHO as adenocarcinoma (papillary, tubular, mucinous, signet ring cell), adenosquamous, squamous cell carcinoma and undifferentiated carcinoma.

Lauren has classified gastric carcinoma as Intestinal and Diffuse types.[16]

In Intestinal type, the incidence of male : female ratio is 2:1 and mean age of detection is 55 years. It commonly presents as an exophytic intraluminal mass with an expansile growth

pattern as it infiltrates the wall and characterised by tubular, papillary and solid microscopic patterns, with mucin being restricted to the glandular lumina.The 5-year survival rate is approximately 20%. It has got an almost 100% association with intestinal metaplasia and Helicobacter pylori. Infection.[3] Dietary and environmental factors are believed to be crucial to the development of intestinal type gastric carcinoma.[3]

In Diffuse type, there is an equal sex distribution and it tends to occur in younger patients with a mean age at diagnosis of 48 years. It commonly presents as an ulcerative, infiltrative tumour with a diffusely infiltrative pattern of growth in the gastric wall and characterized microscopically by poorly differentiated, discohesive cells, often signet ring cell type, and often associated with intra and extracellular mucin. It has got a poor prognosis, with the 5 year survival rate less than 10% and has got a lower association with intestinal metaplasia and Helicobacter pylori infection than for intestinal type of gastric carcinoma. Factors in the causasion of the diffuse type of gastric carcinoma are less strongy related to diet and environment. Genetic factors like late mutation or altered expression of genes coding for basal membrane proteins, pertinent receptors or various adhesion molecules like integrins, cadherins, and catenins are thought to play a greater role.[3,17] As with all schemes, Lauren's classification is not perfect. About 10% of cases will be unclassifiable or of mixed type.

Based on the growth pattern gastric carcinoma is divided into two types by Ming as, Expansile and Infiltrative types.

The expansile type of tumours grow as cohesive cell groups and infiltrative tumours have a diffusely infiltrative edge.[3]

Some authors have tended to equate the terms of intestinal and diffuse type of carcinoma with well differentiated and poorly differentiated tumours. However, this is misleading, as

some poorly differentiated carcinomas may be sharply circumscribed and intestinal in type. Diffuse carcinomas are usually poorly differentiated with regard to gland formation, but some may have low grade nuclear features. Both diffuse and intestinal types are strongly associated with H.Pylori infection. [18,19]

Epidemiology and Pathogenesis of Gastric cancer and precursor lesion:

Adenocarcinoma of stomach is currently the fourteenth leading cause of death in the world.[20] It is primarily a disease of older individuals and is rare under the age of 40 years.

Types of intestinal metaplasia:

Type I: Closely resembles the morphology of small intestine with absorptive enterocytes having well defined brush borders and well formed goblet cells.

Type II: Incomplete metaplasia with irregular mucous vacuoles and absence of brush borders. Absorptive enterocytes are not easily identified. Predominantly secrete sialomucin and occasionally sulphomucin (abnormal mucin)

Type III: Same as type II but predominantly secrete sulphomucin.[21]

Molecular processes contributing to the risk to develop gastric intraepithelial neoplasia and adenocarcinoma with reference to Helicobacter pylori:

Chronic H.pylori infection has been associated with the development of gastric neoplasia and adenocarcinoma. Two bacterial virulence markers of Helicobacter pylori, the cytotoxin associated gene product (cag A) and the vacuolating cytotoxin (Vac A) may play a major role in determining the clinical outcome of Helicobacter infections.The cag A, a 120-145 kilodalton protein, localized at one end of the cag pathogenicity island (cag PAI), a 37-kilobase genomic fragment. It contains 31 putative genes and is associated with more severe outcomes.This stretch of DNA, codes for a type IV secretion (TFESS) apparatus used to inject bacterial protein such as CagA into host epithelial cells.[20]

In addition to the widely recognized association of gastric cancer with the cag A+ genotype and phenotype of H.pylori, vacS1, and vacAm1 genotypes have also been implicated as markers of a particularly strong association.[22] The increased risk of developing gastric cancer is due to the hyperproliferation of gastric epithelial cells induced by H.pylori. In vitro infected epithelial cells , and in vivo studies in humans and animal models have been used to examine bacterial factors involved in the hyperproliferative response in the gastric epithelium.

The Cag PAI [23,24,25] and host genetic polymorphisms in the interleukin-Iβ (IL-1β) and IL-1 receptor antagonist genes are associated with overexpression of IL-1 and hypochlohydria.[26,27] have been linked to an increased risk of developing intraepithelial neoplasia and intestinal type of gastric cancers.

Epithelial proliferation indices have been positively correlated with the degree of histological inflammation in the gastric mucosa in H.pylori infected patients [28,29,30] and patients with H.pylori negative gastritis do not have increased gastric epithelial cell proliferation compared to uninfected controls.[31,32,33] A significant decrease in gastric epithelial cell proliferation has been observed following successful eradication of H.pylori.[32,33]

H.pylori and inflammation:

Inflammation is a critical component of tumour progression and cancer could arise from sites of infection and chronic irritation. H.pylori is a genomically diverse pathogen[34] and several bacterial virulence factors are considered to have a key role on the epithelial response to infection. Only H. pylori genes containing the 40kb cag PAI[35,36] trigger signalling cascades in the gastric epithelial cells resulting in the release of proinflammatory cytokines and chemokines involved in the immediate early response transcription factors AP-1 and NF-kB.[37]These transcription factors contribute to the activation of proinflammatory CXC chemokines, which invivo, attract neutrophils towards the colonized epithelium and other innate defences. Of particular interest has been the observation that chemokines such as IL-8 are upregulated in gastric epithelial cells by cag PAI positive H.pylori strains.[38,39] H.pylori stimulates the transcription factor NF-kB which involves the activity of the kinases IKKα and IKKβ.[40]AP-1 activation involves C-terminal Janus-kinase(JNK) activity.[41] The bacterial effector injected by the cag PAI type IV secretion system is peptidoglycan that is recognized by the intracellular nucleotide-binding oligomerization domain protein (Nod1) receptor molecule [42] which directly activates NF-kB. Nod1 belongs to a family that includes multiple members with Nod and leucine-rich repeats and recognizes peptidoglycan derived primarily from Gram negative bacteria.[41] Thus certain signalling cascades that lead to the activation of the IKK complex, JNK kinase and p38kinase, are only activated by H.pylori strains carrying the active cag PAI.[43] The cag PAI encoded Cag A protein, which is translocated into the gastric epithelial cell via the type IV secretion system,[44,45,46] is indispensable for H.pylori-induced NF-κB activation.

Activation of proliferation-Associated signalling in H.Pylori infection:

Whilst clinical and animal model studies have investigated several aspects of the bacterial induced hyperproliferative resposes, recent in vitro studies with gastric epithelial cells have begun to delineate the importance of specific signalling pathways. Furthermore, the contribution of these pathways to over expression of key genes potentially involved in gastric neoplasia has been examined.

The epidermal growth factor receptor (EGFR) and related EGFR ligands are thought to have an important role in gastric mucosal repair. [47] Studies have demonstrated that H. pylori activates the EGFR in gastric epithelial cells.[48,49] The upregulation of HB-EGF gene transcription by H. pylori requires metalloprotease, EGFR and MEK1 activation.[49] EGFR transactivation and increased expression of HB-EGF in gastric epithelial cells is induced by both cag PAI-positive and cag PAI-negative H. pylori strains.[49] H. pylori infection in humans is associated with increased gastric mucosal levels transcripts.[50] Recent in vitro studies indicate that H. pylori induces the receptor tyrosine kinase HER2/Neu (ErbB-2), another member of the EGF receptor family, in gastric epithelial cells.[51] Gastric expression of EGFR ligands amphiregulin.[52,53] and HB-EGF[54,55] are also increased in patients with H. pylori infection and/ or gastric cancer. Additionally, expression of several ADAM metalloprotease disintegrin family genes is strongly increased in gastric cancer mucosa.[56]

Recent data show that H. pylori induces the activation of c-Met and cell scattering (motogenic response) in adenocarcinomatous (AGS) gastric epithelial cells.[51,57] Direct involvement of c-Met in the stimulation of host epithelial cell motogenic response by H. pylori was confirmed by using small interfering RNA (siRNA) to silence the expression of the c-Met receptor by RNA interference (RNAi) in epithelial cells. Compared to the PAI-positive wild-type strain, an isogenic cagA mutant strain and a virB11 mutant strain, lacking a funtional type

IV secretion system induced only a weak motogenic response in AGS cells.[51] Physical interaction of Cag A and PLCγ and activation of PLC γ by H. pylori contribute in the motogenic response. Further, MAP kinase signaling events are critical for the induction of the motogenic response in H. pylori-infected epithelial cells.[51] The observed interaction of the tyrosine phosphatase SHP-2 and phosphorylated CagA[58,59] is of high interest in the context of H. pylori-induced c-Met regulation. Numerous experimental and clinical data indicate a particular role of Hepatocyte growth factor (HGF) and the protooncogene c-Met in tumour invasive growth. Thus, H.pylori-induced c-Met receptor signal transduction pathways could be responsible for cancer onset and tumour progression.

Based on previous studies, wild-type H. pylori strains and the cagA mutant strain could activate Rho GTPases Rac1 and Cdc42 in gastric adenocarcinoma (AGS) cells.[60] Furthermore, Rac1 and Cdc42 are recruited to the site of bacterial attachment.[61] Rho GTPases control polarity, protrusion, and adhesion during cell movement.[62] Thus, during H. pylori infection the activation of Rho GTPases contribute to the motogenic response in host cells.

As in many human tumour cells, gastric cancer cells overexpress COX-2,[63] and induce nitric oxide synthase.[64] COX-2 and prostaglandin E_2 (PGE_2) are implicated in maintaining the function and structure of the gastric mucosa by modulating diverse cellular functions, such as secretion of fluid and electrolytes, and cell proliferation.[65] COX-2 mRNA expression and PGE_2 synthesis in gastric epithelial cells and experimentally infected mice[66] and in human gastric mucosa[63,67,68] has been demonstrated in H. pylori infection, indicating that COX-2 is involved in H. pylori-related gastric pathology. H. pylori-triggered induction of the COX-2 gene appears independent of the cag type IV secretion system, and involves activation of the mitogen-activated extracellular signal-regulated kinases MEK and ERK.[66] A rate-limiting step in the control of PGE_2 is the relase of arachidonic acid (AA) from membrane phospholipids, which is

known to occur via a number of different pathways. H. pylori induces the release of PGE_2 and AA in gastric epithelal cells by activation of the cytosolic phospholipase A_2 via pertussis toxin-sensitive heterotrimeric $G\alpha_1/G\alpha_0$ proteins and the p38 kinase. PGE_2 production via AA release is predominately synthesized from phosphatidylinositol. In contrast to the H. pylori wild-type strain, an isogenic strain with a polar mutation in the cag PAI only weakly activates AA synthesis.[69]

It is generally accepted that H. Pylori is the cause of most cases of chronic gastritis followed by atrophy, intestinal metaplasia, dysplasia and carcinoma.[70,71,72,73,18] In western countries individuals with H.Pylori infection have an active superficial gastritis, most predominant in antrum in which the organisms are easily identified (diffuse antral gastritis). This type of gastritis may result in duodenal ulcer probably as a result of increased basal acid output following a heightened parietal cell response to stimulation.[74] In contrast, individuals infected by H.Pylori in underdeveloped countries typically have atrophic gastritis (multi focal atrophic gastritis) which is patchy in distribution and involves both pyloric and corpus mucosa.[75]

Organisms may be sparse in gastritis, which is associated with peptic ulcer, occurring particularly at the antrum-corpus junction along the lesser curvature.[74] This multifocal atrophic gastritis is also associated with the development of gastric adenocarcinoma.[18,75,76,77]

The contention that the pathogenesis of intestinal type of gastric cancer is a multistep process is supported by the observation that both atrophic gastritis and intestinal metaplasia are found in high incidence in patients with intestinal type of cancer and in countries with a high incidence of gastric cancer.[78]

In 1994, WHO, classified H.Pylori as grade I human carcinogens.[13]. Why some patients

develop gastric atrophy while others do not progress beyond superficial gastritis is not known.[79]

In superficial gastritis, H.Pylori organisms are in direct contact with surface and foveolar epithelial cells but not with cells in the glands. The bacteria are able to bind to class II major histocompatibility-complex molecules on the cell surfaces, inducing apoptosis.[75]

H.pylori induced cell cycle control and apoptosis:

Exposure of epithelial cells to H.pylori alters cell proliferation rates and apoptosis invitro and invivo. Invitro studies have demonstrated that cyclinD1,[80] expression induced in H.pylori infected epithelial cells is partly dependent on Cag pathogenicity island. CyclinD1 regulates passage through the G1 phase, and Cyclin D1 overexpression shortens the G1phase and increases the rate of cellular proliferation. CyclinD3 is frequently detected in the antral mucosa of H.pylori infected patients[81] and Cyclin D2 over expression, together with reduced p27kip1 expression, are closely associated with H.pylori infection and intestinal metaplasia.[82,83] Further, expression of the intestine specific homeobox gene CDX2 has also been observed in patients with chronic gastritis and is also closely associated with intestinal metaplasia.[84] CDX2 plays an important role in differentiation and maintenance of intestinal epithelial cells. Presumably in the progression to neoplasia in the human gastric mucosa, apoptosis in epithelial cells decreases but proliferation increases.

H.pylori triggers apoptosis via a Fas dependent pathway, which depends on the expression of the Cag pathogenecity island,[85] whereas activation of the nuclear hormone transcription factor peroxisome proliferators activated receptor gamma (PPARγ) suppresses H.pylori induced apoptosis, which depends presumably on the ability of PPARγ to inhibit H.pylori activation of NF-kB. Damage may be mediated by translocation of Cag A protein into epithelial cells.[86]

In addition, enhanced mucosal levels of interleukin, especially IL-8, and tumour necrosis factor are mediated through Cag-A producing strain of H.Pylori.[75]

H.Pylori also induces a vigorous systemic and mucosal humoral response.[75] Upto 80% of patients infected by H.Pylori may have autoantibodies directed against the canalicular membranes of parietal cells, or the luminal membrane of foveolar epithelium.[87] Moreover, the canalicular antibody has H+/K+ adenosine triphosphatase specificity.[88] Damage to the epithelium may occur because of this antigenic mimicry where circulating antibodies or lamina propria T lymphocytes are the triggers for parietal cell destruction.

Acute gastritis due to H.pylori is rarely seen in biopsy material because the infection is usually either asymptomatic or accompanied only by minor gastrointestinal discomfort. The histological features include conspicuous pit abscess and exudation of neutrophils into the surface epithelium. This is accompanied by marked epithelial degeneration and regeneration which may be syncitial in type.[89,90]

Diffuse chronic H.pylori gastritis predominantly affects the pyloric antral mucosa, and although inflammatory changes are generally scanty in the corpus, organisms may be found in the surface mucus in all areas including the cardia.

The mucosa shows a dense infiltrate of chronic inflammatory cells, in which plasma cells are especially prominent. In the corpus mucosa, any inflammation present is confined to the superficial zone. In the pyloric mucosa, inflammation is predominantly superficial but may also involve the full thickness of the mucosa and may surround and separate the glands without causing atrophy. Lymphoid follicles with germinal centers are usually seen, particularly in the deeper portion of the mucosa. This finding, which is easy to identify on low power microscopic

examination of biopsies, is virtually pathognomonic of presence of H.pylori.[91]

Evidence for the role of host genetic factors:

Host genetic factors contribute significantly to the clinical outcome of H.pylori infection There is emerging evidence of host genetic factors that control both the host's innate immune response and its inflammatory response against H.pylori infection. There is an important interaction between these host genetic factors and H.pylori virulence factors which contribute to the mucosal damage and physiological abnormalities that increase the risk of cancer and its precursors. In the the host, there are functional polymorphisms in the interleukin-1 gene cluster [26,27] and tumour necrosis factor alpha genes (TNF-α-308) [92] which will increase the risk of non cardiac gastric cancer but not the other upper gastrointestinal malignancies,[93] and the risk seems to be significantly increased in the presence of proinflammatory genotypes of IL-1 and of H.pylori virulence strains 21 in some geographical areas. The risk applies to both intestinal and diffuse type of gastric adenocarcinoma.[94]

Pathogenesis of Helicobacter infection:

The organisms are Gram negative, slender, measuring about 0.5to0.9x2.5to3.0 μm size. They are seen as curved spirals in the superficial mucous layer where they tend to be attached to the epithelium at the site of intercellular junction.[95] Genetic factors contribute significantly to the clinical outcome of H.pylori infection. There is an important evidence of host genetic factors that control both the host's innate immune response and its inflammatory response against H.pylori infection. The interaction between host genetic factors and H.pylori virulence factors will contribute to the mucosal damage and physiological abnormalities that increase the risk of cancer and its prevalence.[96]

The bacteria are easily seen on H&E, if the density of organism is high. If density of organisms is low, Giemsa stain , Warthin Starry stain, Modified Steiner Silver stain, Acridine orange fluorescent stain, Leung stain,, Genta stains [97,98] Immunohistochemical methods [99] can be used to detect H.pylori. With the use of these techniques, H.Pylori has been found in 90% of patients with chronic gastritis, 95% with duodenal ulcer disease, 70% with gastric ulcer and 50% with gastric cancer.[100] Occasionally, following treatment with proton pump inhibitory drugs, H.Pylori can be present in the stomach as coccoid forms.[101] These are solid, round, basophilic, dot like structures on routine histology.

Ultrastructurally, they are 'U' shaped with the ends of the two arms joined by a membranous structure. Coccoid forms generally coexist with spiral forms but can be reliably identified only by specific immunohistochemical staining.[102]

Cultural characteristics:

Helicobacter pylori produces enzymes like Oxidase, Catalse, and Urease. When cultured on supplemented blood agar (37◦C), the colonies usually take 3 to 5 days to appear and they are circular (1-2mm), convex and translucent in appearance. There is slight haemolysis in blood agar around colonies which are grayish in colour. H.pylori is microaerophillic [grow in air with enhanced Carbondioxide (10%) or mixture of CO2/O2/N2]. Media for primary isolation include Skirrow's medium with vancomycin, polymyxin and trimethoprim, and chocolate medium.

Flagella:

H.pylori has multiple flagella at one pole. The flagella are sheathed with a covering that is continuous with the outer membrane components of the body wall. The

organisms are actively motile. The motility is best demonstrated in broth culture. They exist in two forms, spiral and helical. Each flagella is about 30 nm diameter with a filament of 12-15 nm and have distinct terminal bulbs.

Electron microscopy also reveals the presence of a 40 nm thick glycocalyx or capsule like polysachharide rich layer external to the cell wall unit membrane. It undergoes a morphological change from bacillary to coccoid form with an associated loss in the culturability. Coccoid form may revert to an infectious bacillary form under appropriate conditions.

Biochemical characteristics:

H.Pylori produces catalase, cytochrome oxidase but is the most notable for urease and alkaline phosphatase activity. Typical strains are positive for alkaline phophatase, acid phosphatase, leucine arylamidase, naphthol AS-B1-phophorylase esterases. An important difference between strains is their ability to produce a vacuolating cytotoxin in human and animal cell lines.

Antibiotic sensitivity:

About 80% of H.pylori are susceptible to Cephalothin (30mg disk). About 95% of H.Pylori are resistant to Polymyxin B. (300 IU disk)[103] and 80% of H.Pylori are resistant to nalidixic acid (30mg disk).

Macromolecular characteristics:

Genomic DNA:

The genomic DNA is a single circular molecule with a mean size of 1.71 Mb (range-1.40 to 1.73 Mb) and with a base composition in the range of 35-37 mol% (G+C).

Fatty acid composition:

The major cellular fatty acids are tetradecanoic and cis- 11,12 methylene octadecanoic and with smaller amounts of hexadecanoic acid and 3 hydroxy decanoic acid. The main respiratory quinine is Menaquinone-b (MK-6). Extrachromosomal DNA- plasmid DNA is present in about 45% of the strains.

Lipopolysaccharide (LPS):

The lipopolysaccharides express Lewis and Y group antigens.

H.Pylori- virulence faqctors: Role of Cag A and Vac A

Among people infected with H.Pylori, the virulence of the infecting strain is a major determinant of development of the disease. Strains producing Vaculolating cytotoxin A activity (vacAs1,vacAm1 genotypes),[22] are more commonly isolated from people with peptic ulcers than without it.[104,105] Infection with strains possessing cytotoxin associated gene product A (Cag A) is more common among people with peptic ulceration and gastric adenocarcinoma than without it.[23,106]

Cag A is the marker for Cag A pathogenecity island, which includes genes necessary for the enhanced inflammation induced by pathogenic strain. Serological detection of infection with Cag A+ strains is at present the best potential test for virulence. However, before strategy of screening and selective treatment can be considered, it is important to assess whether Cag A-ve strains are entirely non pathogenic.

Other genteric determinants implicated in the virulence of H.Pylori are Flagellin genes

(fla A, fla B), Urease gene cluster, Ure A, and Ure B which encode structural subunits, Ure E,F,G,H and I for urease activity, Pic B which induces the production of IL-8 by gastric epitheial cells.

Epidemiology of Helicobacter pylori : [95]

Epidemiology of H.Pylori infection has been extensively studied worldwide especially in USA, UK and orientals. The prevalence of H.pylori in otherwise healthy individuals varies depending upon age, socioeconomic class, and country of origin. The infection is usually acquired in childhood. In developing countries children are typically infected by 10 years, whereas in developed countries there is an age related increase in prevalence.

Age:

Most H.Pylori infections occur in childhood and in developing countries many children are infected by the age of 10 years. Since most adults have already acquired the infection in childhood, only about 0.3 – 0.5% of adults develop new infections each year.[95]

Socioeconomic status:

Low Socioeconomic status is the major risk factor (especially in childhood) and close personal contact, overcrowding, poor sanitation are risk factors.

Genetic factors:

Recent studies of twins and children of different races suggest a genetic predisposition to H.Pylori[95] .

Sex:

Seroprevalence is often similar in males and females.

Occupation:

Increased H.Pylori seropositivity has been seen in gastroenterologists and endoscopists. Submariners showed a higher rate of H.Pylori. seropositivity than in other military groups.[95]

H.Pylori habitat and mode of spread:

Natural Habitat is human stomach especially in the mucous secreting epithelium of the gastric antrum.

Other sites:

Areas of gastric metaplasia and ectopic gastric mucosa in other parts of GIT. H.Pylori has been detected in dental plaque, saliva and faeces by PCR.

Mode of survival of H.Pylori in the gastric antrum:

The bacteria live in and beneath the mucous layer that covers the gastric mucosa. The ability of H.pylori to bind specifically to gastric type epithelium is termed Tissue tropism, a property that prevents the organism from being shed during cell and mucous turnover. Tight attachment of the fibrillar adhesion on the bacterium to the carbohydrate receptor on the mucosal cell results in the formation of attaching-effacing lesion (adherence pedestal) which inturn leads to actin polymerization and possibly epithelial cell distruption.[95]

It colonises the surface of the gastric mucosa and may extend into the gastric glands. Spiral shape and motility enables it to resist peristalsis. The enzyme urease produced by the organisms converts urea to ammonia which enables the organisms to survive in the mucosa despite the high acidity of the lumen. The micro-aerophilic nature of the organism is suited to the environment of the mucosal gel and specific adhesions produced by the organisms are responsible for long-term survival.

Transmission:[95] .

Potential transmission is mainly by man

1. Faeco-oral
2. Oral – oral
3. Gastro-oral.

Faeco –oral transmission:

Isolation of H.Pylori DNA from faeces and from drinking water by PCR analysis supports this mode of transmission.

Oral-oral transmission:

Evidence of isolation of H.Pylori from dental plaque and saliva by culture and PCR supports this mode of transmission.

Gastro-oral transmission:

This may be common in children where reflux and vomiting are common. A physician became infected with H.Pylori after giving mouth-mouth resuscitation to a H.Pylori +ve patient who had recently vomited [95]. Iatrogenic person-person transmission via endoscopes has been reported and high prevalence of infection among endoscopists particularly those who do not use gloves, suggests that transmission occurs through instruments contaminated with the gastric secretions.[95]

H Pylori & Gastric cancer:

Gastric cancer is the cause of more than 7,50,000 deaths annually. Recently, the importance of H.Pylori has been recognized. The IARC (International Agency for Research on Cancer) has accepted H.Pylori as Grade I human carcinogen based on ecological correlation

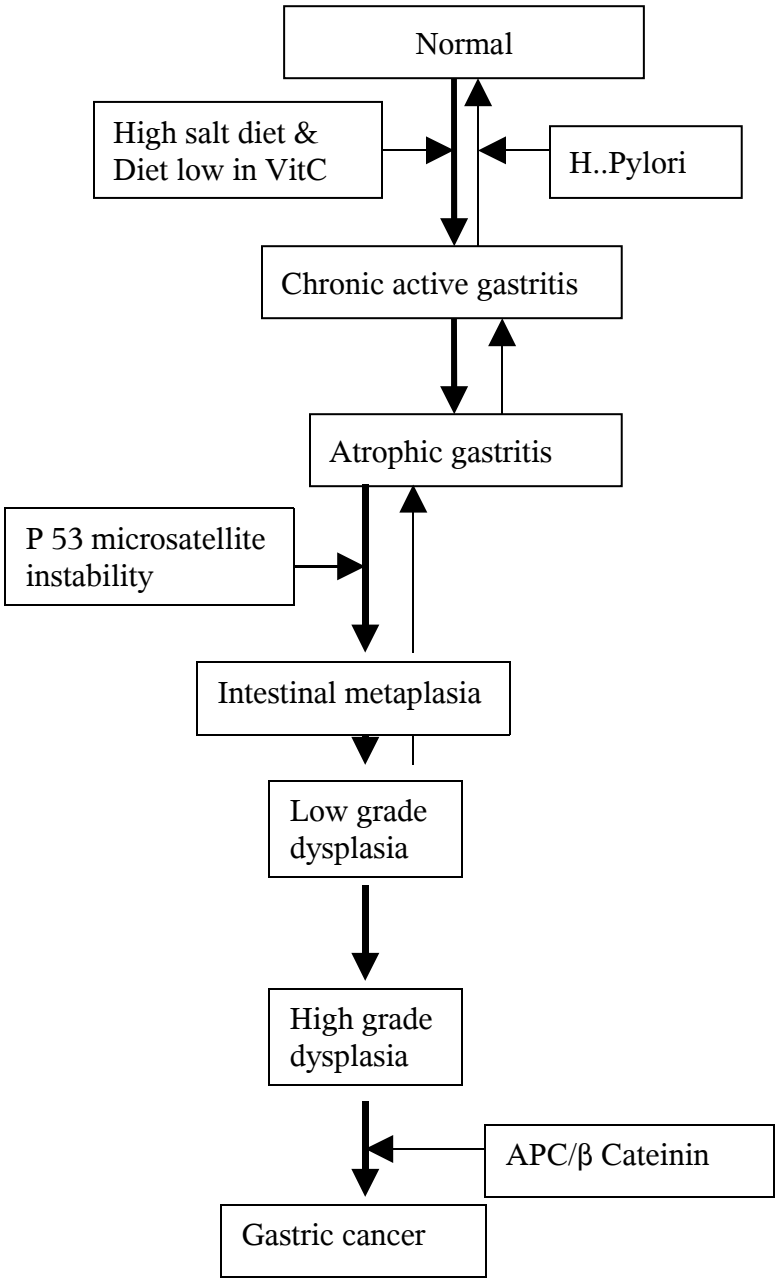
studies such as Eurogast study and other case-control studies.[107]

As the infection is rarely self limiting, it initiates sequence of inflammatory mediated changes within the gastric epithelium causing first acute and then chronic gastritis. And over a period of decades, it causes increasingly degenerative changes and evolution towards well established precancerous condition of atrophic gastritis, metaplasia and dysplasia, in case of intestinal type of gastric cancer. Currently, it remains uncertain whether the diffuse type of gastric cancer follows a similar histopathogenesis. One recent estimate shows that 53% and 60% of gastric cancers in the developing and developed world respectively can be attributed to H.Pylori.

Genetic factors for gastric cancer:

Most common genetic abnormality found in gastric cancer tends to be loss of heterozygosity [LOH] of tumour suppressor gene p53. Initial studies found that LOH (60-70%) and mutation (38% to 70%) of p53 gene are quite common in gastric cancer. In addition, p53 mutations are also present in intestinal metaplasia (38%) and gastric dysplasia (58%)[108.] Further evidence for the role of p53 in the early stages of gastric cancer comes from studies in mice, which are homozygous for p53, which exhibit an increased proliferative response to Helicobacter infection compared with wild type mice.[109.] Increased proliferation has generally been shown to correlate with an increased risk of developing gastric cancer.[31]

Gastric carcinogensis – Hypothetical sequence:



The proposed multistep pathway in the pathogenesis of gastric cancer shows that infection with H.pylori may be a common initiating event, whereas genetic alterations (p53,APC/Beta catenin pathway) or Microsatellite instability may play a role. Thin arrows represent steps that are potentially reversible.[95]

The overall survival rates for gastric cancer are poor and depend on the stage of disease at

the time of presentation.[110] In Japan where small intramucosal lesions can be detected endoscopically during screening examinations, trans endoscopic gastric mucosal resections result in improved survival.[111] Unfortunately these types of aggressive screening and prevention programs are not universal and the disease typically presents at later stages, resulting in a poor prognosis with overall 5 year survival rates of less than 25%. Surgical resection is the only curative approach for advanced gastric cancer. A study suggests that adjuvant chemotherapy is beneficial in those patients who undergo a curative resection.[20] However the median survival for these patients still remains less than 4 years. Thus it is clear that prevention is the best overall approach to the treatment of gastric malignancy.

It is now more than 10 years since the discovery of H.pylori as a gastric carcinogen.[13] This has led to effective eradication strategies. Effective eradication along with a natural decrease in infection because of improved living conditions has resulted in decling gastric cancer rates in Western countries, although this still remains a significant cause of morbidity and mortality in other parts of the world.

Gastric MALT-LYMPHOMA and H.pylori: [110]

The close association between H.pylori and gastric MALT lymphoma is beyond doubt and is seen in 72-98% of low grade cases. H.pylori is less commonly found in high grade gastric lymphomas being seen in 71% of cases with concomitant low grade component.[110] H.pylori infection is only seen in approximately 51% of individuals with secondary involvement of stomach by lymphoma of the nodal type.[110] Lehours et al has discovered that apart from cag A,vacAm1 and vacAs1 genotypes other virulence patterns like HopZ play a role in the development on gastric B-cell lymphoma. In addition to histomorphological studies, recent epidemiological,[32,20] molecular, biological and experimental data clearly indicate that

H.pylori plays a decisive role in the development and progression of gastric MALT lymphoma. This convincing evidence inevitably involved a therapeutic effort. In 1993, Wotherspoon and colleagues[112] reported complete regression of low grade lymphoma following successful eradication of H.pylori in 5 out of 6 cases. Similarly in a large prospective series of 90 cases, after exclusive H.pylori eradication 62% of patients showed complete regression and 12% had partial remission in a follow up period of 12-89 months offering a real chance of cure.[110] Undoubtedly, eradication of H.pylori represents a fascinating therapeutic option because of its simplicity and efficacy.

H.PYLORI AND CLINICAL ASSOCIATIONS: [113]

H.pylori is implicated in the following diseases like Duodenal ulcer, Gastric ulcer, Gastricadenocarcinoma, Gastric MALT lymphoma, Duodenitis, Barrett's oesophagus, Bronchiectasis, Anorexia of aging, Sudden infant death syndrome, Iron deficiency anaemia, Cerebrovascular disease, Hypertension, Migraine, Vomiting of pregnancy, Autoimmune thrombocytopenic purpura, Hyperammonemia, Growth retardation, and chronic utricaria.

Methods of diagnosis of H.pylori:

Different invasive and noninvasive diagnostic tests are available for the diagnosis of H.pylori. Though far from an exhaustive coverage the main points of each method are given below.

In the Invasive method, endoscopic biopsy is done and small bits of tissue are obtained and they are subjected to the following tests namely, the Rapid urease test, Culture, Histology,

and PCR.

The noninvasive tests are namely, the Antibody detection in serum, Urea breath test, and the Stool antigen test (HpSA test).

Rapid urease tests:

Rapid urease test is a simple biochemical test which involve placing endoscopy biopsy specimen into a small amount of solution containing urea, pH indicator (phenol red) and bacteriostatic agent. If H.pylori are present, bacterial urease hydolyzes the urea and produces ammonia. Alkalinisation of the medium produces a colour change from yellow to pink. Results are read between one minute and 24 hours. The sensitivity and specificity of these methods are 90% and 100%.[95] Two other RUT are Hp test and Pyloritek-skip test. Interpretation may be performed within one hour.

Culture:

This is not used routinely, but one major advantage is the detection of antibiotic sensitivity and culture is indicated when antibiotic resistance is suspected. The culture medium used is Brain Heart Infusion (BHI) agar supplemented with 7% lysed horse blood.

Selective media are BHI agar supplemented with 10% sheep blood, polymyxin B, Vancomycin, trimethoprim and amphotericin B and Skirrow's selective medium. Incubation periods of upto 10 days are usually required in order to optimize the culture isolation rates especially in post treatment settings.

Histopathology:

The **gold standard** for the diagnosis of H. pylori is the detection of the organism in the

gastric biopsy specimens.

Stains commonly used are:

Haemotoxylin-eosin

Giemsa

Warthin-Starry

Acridine orange

Browns-Hopps

PCR :

This is the most sensitive technique for the detection of microorganisms and useful for posttreatment diagnosis of H.pylori when the bacteria may be very low in number. False positive results are high due to inadequate cleaning and disinfection of endoscopes or from cross contamination in the laboratory.

Non Invasive tests :

Antibody detection test:

Seological tests detect the presence of H.pylori IgG antibodies, by ELISA, and LATEX agglutination tests. The Sensitivity is 91% and Specificity is 97%.[95]

The advantages are, that they are non invasive, inexpensive and avoids the pitfalls, inherent in methods employing gastric biopsies or even urea breath tests.

Urea Breath Tests:

^{13}C and ^{14}C Urea Breath Test (UBT) – measures the activity of H.pylori urease. Patient

ingests ^{13}C and ^{14}C labelled urea. If H.pylori is present in the stomach, urease hydolyses the labelled urea releasing labeled bicarbonate transported in blood to the lungs and exhaled as Carbondioxide (CO2).

The breath is collected and ^{13}C or ^{14}C is measured. This test is preferred in children and in pregnant women. It should not be used in patients who has taken Proton Pump Inhibitors, bismuth compounds or antibiotics.

Other assays include:

1. Detection of antibodies in the urine
2. Measurement of labelled serum bicarbonate following administration of ^{13}C urea as in UBT.
3. Measurement of ^{14}C in the urine
4. Measurement of 15-n labelled ammonia which is absorbed and excreted in the urine.

Sensitivity of Urea Breath Test is 90% and specificity is 96%.[95]

Stool antigen test:

This is a rapid non invasive, easy to perform test can be used to detect active infection, monitor effectiveness during therapy and to confirm cure after antibiotic use. Comparative accuracy, availability, and cost of tests for H.pylori infection puts the sensitivity and specificity of the stool antigen test as 90-95%.[114]

MATERIALS AND METHODS

Study design:

The study was carried out in the Department of Pathology, Govt. Stanley medical college, with the help of Dept. of Medical and Surgical Gastroenterology, Govt. Stanley medical college hospital, during the period 2005 to 2007. A total of 60 gastrectomy specimens with carcinoma stomach were received. Out of this, a random sample of 50 gastrectomy specimens were taken for this study.

Method:

For all the 50 cases, details of age, sex were recorded. Depending upon the site of growth, the stomach was opened through the greater or the lesser curvature. The presence of growth was noted. The gross appearance and three dimentional measurement were taken. The site of the lesion were also taken and recorded.

Multiple bits from the lesion and the adjacent areas were taken and processed routinely for paraffin embedding. 4µ sections were taken and stained by H&E. Sections were further stained by Giemsa stain.

Sections were also mounted on chrome alum coated slides.

The neoplasm was subtyped as well differentiated, moderately differentiated and poorly differentiated carcinomas under H&E.

Giemsa stained sections were used for recording the presence of Helicobacter pylori.

Sections mounted on chrome alum coated slides were used for IHC.

GIEMSA STAINING TECHNIQUE. [115]

Giemsa stock solution:

Giemsa stain powder - 4 gm

Glycerol - 250 cc

Methanol - 250 cc.

The powder is dissolved in glycerol at 60°C with regular shaking. Methanol is added, the mixture is well shaken and then allowed to stand for 7 days. Filter before use.

Working Giemsa stain:

Giemsa stock solution - 4 cc

Buffered distilled water - 96 cc

Method:

1. Dewax in Xylol, hydrate through graded alcohols to water
2. Rinse in buffered distilled water (pH 6.8)
3. Stain in working Giemsa stain overnight
4. Rinse in distilled water
5. Rinse in 0.5 aqueous acetic acid until the section is pink.
6. Dehydrate, clear in xylene and mount in DPX .

Preparation of gelatin coated slides:

Chrome alum - 0.05 gm

Gelatin - 0.3 gm

Distilled water - 100 ml

First chrome alum is added to distilled water and then the distilled water is heated to 60°C. Gelatin is added slowly to the heated distilled water. Glass slides are then dipped in this

solution and dried overnight.

Preparation of Tris Buffered Saline (TBS): 0.005 M TBS

Distilled water	-	10 litres
Sodium Chloride	-	80 g
TRIS (Hydoxymethylamine)	-	6.05 g
1 M Hcl	-	44 ml

Final pH is adjusted to 7.6 with either 1 M Hcl or 0.2 M Tris solution

Preparation of CITRATE buffer solution (antigen retrieval solution):

Trisodium citrate	-	2.94 gm
1N Hcl	-	5 ml
Distilled Water	-	1000 ml

Final pH is adjusted to 6.0 with 1N Hcl.

Antigen Retrieval:

The slides are placed in citrate buffer in the coplin jar and capped. The jar is then heated in a 750 W domestic microwave oven for 15 minutes (5 minutes in low power(40), 5 minutes in medium power(60) and 5 minutes in full power(80) pausing only to top up the fluid.

Procedure adopted for IHC:[116]

1. Dewax the sections in xylene (1/2 hour, two changes) and bring sections to distilled

water.

2. Antigen retrieval using TBS by Microwave oven heating
3. Cool to room temperature in running tap water for 20 minutes.
4. Bring sections to TBS for 5 minutes.
5. Drain and wipe off excess TBS around sections
6. Incubate in endogenous peroxidase blocking reagent for 15-20 minutes
7. Gently wash the slides in TBS for 5 minutes.
8. Wipe off the excess fluid and Incubate in power block for 15-20 minutes.
9. Wipe the excess fluid and incubate in Primary Antibody for 60 minutes
10. Repeat steps 4 and 5
11. Incubate in super enhancer for 30 minutes
12. Repeat steps 4 and 5
13. Incubate in S S label (secondary antibody) for 30 minutes
14. Repeat steps 4 and 5
15. Incubate in DAB (Diaminio Benzidine) substrate solution for 2-10 minutes

(To prepare DAB substrate, add 1ml of Substrate buffer, 1 drop of liquid DAB, and 1 drop of Substrate DAB).

Wash in distilled water, counter stain with Haematoxylin, clear in xylene and mount with DPX.

RESULTS AND OBSERVATION

The collected details of cases have been recorded in the master chart. All collected data were analyzed and following results were observed

H.pylori in carcinoma stomach patients:

Table - 1

Total number of patients included in the study was 50.Out of 50 patients, 25 patients were Helicobacter pylori positive and the rest of 25 patients were H.pylori negative.

Total No. of patients	H.pylori +ve	H.pylori -ve
50	25	25

The prevalence of H.pylori among patiens who were diagnosed as adenocarcinoma stomach was 50% .

Male:female ratio of adenocarcinoma:

Table - 2

Out of 50 cases of adenocarcinoma stomach, 39(78%) were males and 11(22%) were females with a male to female ratio of 3.5:1

Total no of patients	Males	Females
50	39(78%)	11(22%)

Male:female ratio of H.pylori infection:

Table - 3

Out of 39 male patients, 17(43.6%) were H.pylori positive and out of 11 females, 7(63.6%) were H.pylori positive.

Sex	No. of patients	H.pylori -ve	H.pylori+ve
Male	39	22(56.4%)	17(43.6%)
Female	11	4(36.4%)	7(63.6%)

Age incidence of H.pylori:

Table - 4

The age of the patients included in this study ranges between 30 and 75 with mean age of 51.75 years. The incidence of H.pylori increases with age reaching a peak between 41-50 years which is statistically significant with a p value of 0.033.

		H.pylori			
		Negative		Positive	
		n	%	n	%
Age	31-40	2	8.0%	3	12.0%
	41-50	11	44.0%	12	48.0%
	51-60	7	28.0%	7	28.0%
	>60	5	20.0%	3	12.0%

	Age
Chi-Square(a)	8.760
Asymp. Sig.	.033

Mean Age for Subtypes of Gastric carcinoma:

Table - 5

Most of the H.pylori +ve patients in our study are in the age group of 41 to50 years, with mean age of 53 years for well differentiated adenocarcinoma and 47.29 years for poorly differentiated adenocarcinoma .

H.pylori Status	**Grading**	**Number**	**Mean**	**Std. Deviation**	**ANOVA F-test**
Negative	WD	6	54.33	4.320	F=0.53
	MD	4	48.50	10.630	P=0.59
	PD	15	54.00	11.180	
	Total	25	53.20	9.764	Not significant
Positive	WD	11	53.00	10.894	F=0.63
	MD	7	50.45	7.141	P=0.54
	PD	5	47.29	9.196	
	Total	25	50.28	9.388	Not significant

WD-Well differentiated, **MD**-Moderately differentiated,

PD-Poorly differentiated

H.pylori and site of growth:

Table - 6

Out of 50 cases taken for this study, 41 cases were located in the antropyloric region and. Among these 41 cases, 20 cases were positive for H.pylori (48.8%)

Out of the 9 cases, which were located at the body and fundus region ,5 cases were positive for H.pylori(55.6 %)

		H.PYLORI				Chi square test
		Negative		Positive		
		n	%	n	%	
Site of the tumour	Antrum and pylorus	21	51.2%	20	48.8%	χ^2=0.14 P=0.71 Not significant
	Body and fundus	4	44.4%	5	55.6%	
Group	Total	25	50.0%	25	50.0%	

n - number of cases

H.pylori and grading of adenocarcinoma stomach:

Table -7

Out of 25 H.pylori+ve adenocarcinomas, 13 (52%)were Well differentiated, 5 (20%)were Moderately differentiated and 7 (28%) were Poorly differentiated adenocarcinomas. This implies that, H.pylori is more associated with well differentiated adenocarcinoma than poorly differentiated adenocarcinomas.

		H.PYLORI				Chi square test
		Negative		Positive		
		n	%	n	%	
Grading	WD	6	24%	13	52%	$\chi 2$=5.69 P=0.05 significant
	MD	4	16%	7	28%	
	PD	15	60%	5	20%	
Group	Total	25	50.0%	25	50.0%	

WD-Well differentiated, **MD**-Moderately differentiated,

PD-Poorly differentiated

In the case of 25 H.pylori –ve adenocarcinomas, about 15 cases(60%) were poorly differentiated, 4 cases(16%) were moderately differentiated and 6 cases(24%) were well differentiated.

Detection of H.pylori with GIEMSA STAIN:

Table - 8

Out of 50 cases H.pylori was detected in 21 cases using Giemsa stain with 42% of positivity.

GIEMSA	No. of patients	Percent
Negative	29	58.0
Positive	21	42.0
Total	50	100.0

Detection of H.pylori with polyclonal antibodies against H.pylori antigen by Immonohistochemistry :

Table - 9

Out of 50 cases, IHC detected H.pylori in 25 cases (4 cases more than GIEMSA) with a positivity rate of 50%

IHC	No. of patients	Percent
Negative	25	50.0
Positive	25	50.0
Total	50	100.0

Comparison of IHC and GIEMSA:

Table - 10

Out of 50 cases, IHC detected H.pylori in 25 cases with a positivity of (50%) With 4 cases more than in Giemsa stain

		GIEMSA	
		Positive	**Negative**
IHC	Positive	21	4
	Negative	0	25

	ESTIMATE	95%CI
SENSITIVITY	100%	83-100%
SPECIFICITY	86%	68-96%
POSITIVE PREDICTIVE VALUE	84%	63-95%
NEGATIVE PREDICTIVE VALUE	100%	86-100%
k agreement status	k=0.84 and p=0.001	

According to the TWO SAMPLE BINOMIAL PROPORTION TEST,

Z=0.60 and p=0.55

Eventhough statistically there is no difference between IHC and GIEMSA stains, it can be said that upto 29% positivity can be expected more in IHC than in GIEMSA stain.

DISCUSSION

Histological type influences the prognosis of gastric carcinoma. Well differentiated adenocarcinoma and intestinal type of Lauren have the best prognosis and poorly differentiated and diffuse type of Lauren with signet ring cells have the worst prognosis.[3.] The main aim of this study is to know the assiciation between H.pylori and the subtypes of gastric carcinoma (grading) and to confirm the causal relationship between H.pylori and gastric carcinoma, and use of Immunohistochemistry in diagnosing H.pylori in paraffin embedded tissue sections, along with Giemsa stain. Three nested case control studies had shown that infection with H.pylori increases the risk of gastric cancer.(Loin et al[116], Parsonet et al[106], Nomura et al [117]. A combined analysis of these studies has estimated that 73% of gastric cancers are attributable to H.pylori in those who have been infected for over 14 years.

In this study, the percentage of H.pylori positivity in gastric malignancy is 50%. The prevalence of H.pylori among patients who were diagnosed as adenocarcinoma stomach was 50% which correlates with Robey- Cafferty SS et al[118], 1989.

The Male : female ratio of adenocarcinoma in our study is 3.5:1. Okusa et al [119] says that his study has male dominance with male : female ratio of 4.7:1

Most of the H.pylori +ve patients in this study were in the age group of 41 to 50 years, with mean age of 53 years for well differentiated adenocarcinoma and 47.29 years for poorly differentiated adenocarcinoma which coincides with with Enrico solcia et al where the mean age(51.9years) of poorly differentiated adenocarcinoma is lower than that of well differentiated adenocarcinoma.(64.5 years) [17.] The incidence of H.pylori increases with age reaching a peak between 41-50 years which is statistically significant with a p value of 0.033 whereas in the study of Loin et al [116] in which peak age incidence of H.pylori is 60 years.

The location of adenocarcinoma in antropyloric region is 82% (41 out of 50cases) and in the body and fundus region it is 18% (9 out of 50 cases). Though statistically not significant, H.pylori is associated more with adenocarcinoma located at body and fundus region, ie, in 5 /9 cases(55.6%) than tumours located in antropyloric location, ie, in 20/41 cases(48.8%).

Out of 25 H.pylori +ve cases, it is most commoly associated with well differentiated adenocarcinoma with 68.4%(13/25 cases) and least commonly associated with poorly differentiated aenocarcinoma with 20% (5/25 cases) which is statistically significant, with p value of 0.05.

Whereas in the 25 H.pylori –ve cases of adenocarcinoma, about 15 out of 25 cases (60%) were poorly differentiated adenocarcinomas and only 6 out of 25 cases were (24%) well differentiated adenocarcinomas, implying that genetic factors could play a major role in the pathogenesis of poorly differentiated adenocarcinoma.[120.]

When compared with Giemsa stain, Immunohistochemistry has detected 4 cases more (25/50) with a sensitivity of 100% and specificity of 86% and a positive predictive value of 84% and a negative predictive value of 100% and accuracy rate of 92%.

The k agreement status(k=0.84 and p=<0.001) is correlating between the two methods. In this study, out of 39 males,17(43.6%) were H.pylori +ve, and out of 11 females, 7 (63.6%) were H.pylori +ve implying that H.pylori infection is a significant risk factor for adenocarcinoma in women more than in men which coincides with Julie Parsonnet et al [120] which says that H.pylori is +ve in 90.9% in females and 81.6 % in males of adenocarcinoma stomach.

Epidemiological studies have consistently demonstrated an association

between Helicobactor pylori infection and the risk of gastric cancer.An analysis of data from 13 countries showed a strong correlation between the incidence of gastric cancer and the prevalence of H.pylori infection[107]. Prospective serologic studies have reported that persons wih H.pylori infection have a three to six fold higher risk of gastric cancer than the normal persons.[122.] This association seems largely restricted to intestinal type and well differentiated adenocarcinomas and cancers of distal stomach. Because Helicobacter pylori infection appears to be the most consistent factor in gastric cancer induction, the effects of H.pylori treatment on modulation of gastric cancer risk has been evaluated extensively [20].

It has been found that gastric mucosal proliferation is decreased following successful treatment thereby presumably decreasing the frequency of mutation events associated with carcinogenesis.[33,120.] Eradication of H.pylori has been demonstrated to be cost effective as a preventive therapy for gastric cancer if it prevented atleast 30% of cancers.[20.]

In limited studies, H.pyloyi eradication has been suggested to prevent progression and may lead to regression of precursor lesions, such as atrophy.[122] It has also been suggested that H.pylori eradication in patients who have had endoscopic mucosal resection of early gastric cancer can reduce recurrent cancers[20]. A randomized trial was conducted in Colombia and a beneficial effect of H.pylori eradication on atrophy and intestinal metaplasia was observed after 6 year follow up period [123.]

In a clinical trial in China, patients with persistent infection had a 2.1% risk of progression of intestinal metaplasia, whereas H.pylori eradication therapy reduces the risk of progression of intestinal metaplasia significantly.[124.]

The recent data of effects of H.pylori eradication on precancerous lesions as well as the

reduced risk of gastric cancer development strongly support early H.pylori therapy. H.pylori eradication with appropriate antibiotic treatment should be considered in future to prevent malignancy in H.pylori +ve subjects who are at risk for gastric cancer. (members of gastric cancer families).

Screening of gastric cancer patients in asymptomatic persons increase the chance of detecting early cancer and hence may improve overall survival.

Gastric cancer-A PREVENTABLE DISEASE:

H.pylori infection can thus be considered as a substantiative cause of gastric cancer. In H.pylori infection , a stage is reached in which H.pylori is no longer required in the progression of events leading to carcinoma. That is, a point of no return is reached and carcinogenesis can proceed autonomously. So eradication therapy should be started very early at a younger age in order to prevent gastric cancer.

Hence for cancer prevention, asymptomatic individuals should be tested for H.pylori infection and H.pylori positive patients should be treated. (TEST AND TREAT policy).[96].

Test and treat policy is a large scale project , which is clearly expensive and would not be economically cost effective in population where the prevalence rate of gastric cancer in low socioeconomic group is high as in India and Japan. An important priority therefore to be given over the coming years to conduct intervention studies and produce the necessary evidence and thereby prevent gastric cancer which is one of the world's major cancers.

SUMMARY AND CONCLUSION

Fifty gastrectomy specimens of carcinoma received at the Department of Pathology, Stanley Medical College were analysed.

Age, sex and site of the lesion were recorded

Subtyping of carcinoma was done

Giemsa staining was used for detecting Helicobactor pylori

Immunohistochemistry using polyclonal antibodies against H.pylori antigen was also done to assess the role of immunohistochemistry in detection of H.pylori.

Results were tabulated and analysed.

H.pylori was positive in 21 out of 50 cases as per Giemsa and by doing Immunohistochemistry 25 out of 50 cases .

The study revealed the following facts:

1. The peak incidence of adenocarcinoma stomach is between 41 to 50 years with a male : female ratio of 3.5:1

2. The peak incidence of H.pylori positivity in gastric carcinoma is between 41 to 50 years.

3. H.pylori is associated more with well differentiated adenocarcinoma than poorly differentiated adenocarcinoma of stomach.

4. H.pylori is associated in 52% of well differentiated adenocarcinoma, with mean age of 53.45 years, 28% of moderately differentiated adenocarcinoma with mean age of 50

years, and 20% of poorly differentiated adenocarcinoma with mean age of 47.23 years.

5. Sensitivity of Immunohistochemistry is more when compared with Giemsa.

6. Immunohistochemistry can be used to detect coccoid forms of H.pylori especially in screening for the presence of H.pylori in high risk patients and also in patients who had been treated with proton pump inhibitors and to confirm eradication following antibiotics against H.pylori.

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